

IN THE CLAIMS

1. (Currently Amended) A method for regeneration of cotton via somatic embryogenesis with substantially synchronized development of embryos after short duration inositol starvation, said process comprising the steps of:

(i) cutting from the germinated cotton seedling an explant, selected from the group consisting of cotyledon, hypocotyl, mesocotyl, and mixtures thereof;

(ii) culturing the explant for the purpose of callus induction on a first solid medium, on a culture medium containing glucose as the carbon source supplemented with Gamborg B5 vitamins, 2,4-D, BA and inositol, at a temperature between 23 to 33° C in light intensity of at least 90 $\mu\text{mol}/\text{m}^2/\text{s}$ under a 16 hour photoperiod for a period of 3-5 weeks, to enable a dedifferentiated callus to form from the explant;

(iii) transferring the callus from the first solid medium to a liquid medium comprising a basal medium containing glucose as the carbon source and supplemented with Gamborg B5 vitamins and culturing a suspension generated thereof at a temperature from 23 to 33° C in a reduced light intensity of 20-40 $\mu\text{mol}/\text{m}^2/\text{s}$, under a 16 hour photoperiod for a period of time sufficient to form embryogenic clumps;

(iv) screening the suspension through metal sieves of different pore sizes to select embryogenic cells, clumps, or both and subculturing the callus containing somatic embryos to said basal medium;

(v) subjecting the embryogenic cells, the clumps, the callus, or any combination thereof to inositol deprivation, consequent upon subculturing it to a second basal medium devoid of inositol for 8-12 days and then returning the culture to inositol containing medium to enable somatic embryos to synchronize developmentally;

(vi) transferring the somatic embryos to an embryo germination medium on a

support and growing the embryos in embryo germination medium up to the plantlet stage developed sufficiently for transfer to soil as plantlets and;

(vii) further transferring the plantlets to a potting mix for acclimatization and then to field.

2. (Cancel)

3. (Previously Presented) The method as recited in claim 1, wherein the explant is derived from cotton cv Coker 312 and the seedlings are developed by:

- (i) sterilizing cotton seed in a sterilization solution of 0.1% HgCl_2 for 5-10 min.,
- (ii) rinsing the seed in sterile water 4-6 times,
- (iii) scorching the seed in flame of a spirit burner for 5-10 seconds,
- (iv) inoculating the seed on a seed germination medium,
- (v) growing the seed in the seed germination medium in light or dark at a temperature of 23° to 33° C for a period of 6-12 days, and
- (vi) excising the explant from the seedling.

4. (Previously Presented) The method as claimed in claim 3, wherein seed germination medium is a liquid medium comprising salts of Murashige and Skoog and Gamborg B5 vitamins at half of its concentration.

5. (Previously Presented) The method as claimed in claim 3, wherein a carbon source in the seed germination medium is selected from the group consisting of sucrose and glucose at a range of 1 to 3% wt./vol.

6. (Previously Presented) The method as claimed in claim 1, wherein said first solid callus

induction medium comprises following components of Murashige and Skoog medium:

Component	Conc. (mg/L)
a. Salts of Murashige and Skoog medium:	
NH_4NO_3	1650
KNO_3	1900
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	440
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	370
KH_2PO_4	170
KI	0.83
H_3BO_3	6.2
$\text{MnSO}_4 \cdot \text{H}_2\text{O}$	22.3
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	8.6
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.25
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.025
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.025
$\text{Na}_2\text{.EDTA}$	37.3
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	27.8 and
b. Organics	
Myo-inositol	100.

7. (Previously Presented) The method as claimed in claim 1, wherein Gamborg B5 vitamins, wherever included comprise:

Component	Conc. (mg/L)
Nicotinic Acid	1.0
Pyridoxine Hcl	1.0 and
Thiamine Hcl	10.

8. (Previously Presented) The method as claimed in claim 1, wherein 2,4-D as exogenously supplied auxin in first solid callus induction medium is selected from a range of 0.44 to 4.4 μM .

9. (Previously Presented) The method as claimed in claim 1, wherein BA as exogenously supplied cytokinin in first solid callus induction medium is selected from a range of 0.22 μM to 2.2 μM .

10. (Previously Presented) The method as claimed in claim 1, wherein a gelling agent in said first solid medium is selected from the group consisting of agar in the range of 0.6-0.8% wt./vol. and phytigel in the range of 0.15-0.29% wt./vol.

11. (Cancel)

12. (Previously Presented) The method as claimed in claim 1, wherein said explants are cultured on said callus induction medium at a temperature between 23 to 33° C., in light intensity of at least 90 $\mu\text{mol}/\text{m}^2/\text{s}$ under a 16 hour photoperiod for period of not more than 3-5 weeks, to enable dedifferentiated callus to form from any of the explant.

13. (Previously Presented) The method as claimed in claim 1, essentially including the step of transferring callus from the first solid medium to a liquid medium in Ehrlenmeyer flasks at a packing density of 600 to 1000 mg of callus/50 ml of media-and shaking the culture in this and

all subsequent steps until somatic embryos are taken out for germination on a gyratory shaker at 110-130 rpm.

14. (Previously Presented) The method as claimed in claim 1, wherein said basal medium is a basal liquid medium comprising Murashige and Skoog salts, Gamborg B5 vitamins, inositol and glucose as the carbon source.

15. (Previously Presented) The method as claimed in claim 1, wherein a plant cell suspension embryogenic mass and somatic embryos generated thereof in liquid medium are incubated at a temperature from 23 to 33° C., in light intensity of 20-40 $\mu\text{mol}/\text{m}^2/\text{s}$, under a 16 hour photoperiod.

16. (Previously Presented)) The method as claimed in claim 1, wherein said embryogenic mass/clumps are subjected to inositol deprivation for a period of 8 to 12 days, in inositol deprivation medium comprising Murashige and Skoog basal salts, Gamborg B5 vitamins, glucose as carbon source but no inositol, leading to developmental synchronization of somatic embryos.

17. (Previously Presented) The method as claimed in claim 1, wherein said first solid callus induction medium has a pH in the range of 5.4-6.2 and the entire liquid media in said process has a pH in the range of 5.2-5.8, being sterile as a result of autoclaving at 121° C, 16 psi for 16 minutes.

18. (Currently Amended) The method as claimed in claim 1, wherein a potting mix comprises garden soil: sand: Peat moss: vermiculite typically in 2:1:1:1 ratio.

19. (Previously Presented) The method as claimed in claim 1, wherein developmental

synchrony of somatic embryogenesis is utilized for multiplication of an elite cotton cultivar or development of a transgenic cotton cultivar.

20. (Cancel)

21. (Previously Presented) The method as claimed in claim 1, wherein said culture medium and basal medium comprise of Murashige and Skoog medium.

22. (Previously Presented) The method as claimed in claim 1, wherein said period of time sufficient to form embryonic clumps comprises 12-32 days.

23. (Previously Presented) The method as claimed in claim 1, wherein said subculturing the embryogenic callus containing somatic embryos to said basal medium is carried out at intervals of 8-12 days.

24. (Cancel)

25. (Previously Presented) The method as claimed in claim 1, wherein said support for said embryo germination medium comprises vermiculite.

26. (Previously Presented) The method according to part (v) of claim 3, wherein the seed is grown for 9-10 days.

27. (Previously Presented) The method according to claim 15, wherein the plant cell suspension embryogenic mass and somatic embryos are incubated at a temperature from 27-29°C.

28. (Previously Presented) The method according to claim 8, wherein the range is 1.76 to 2.64 μM .

29. (Previously Presented) The method according to claim 9, wherein the range is 0.66 to 1.00 μM .

30. (Previously Presented) The method according to claim 12, wherein the explants are cultured on said callus induction medium at a temperature between 27°C to 29°C.

31. (Previously Presented) The method according to claim 15, wherein the temperature is from 27-29°C.

32. (Previously Presented) The method according to claim 15, wherein the light intensity is 27-33 $\mu\text{mol}/\text{m}^2/\text{s}$.